A CPMAS ^{13}C NMR Study of Molecular Conformations and Disorder of *N*-Octylhexonamides in Microcrystals and Supramolecular Assemblies

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Abstract: Solid molecular mono- and bilayers in bulk aqueous media are usually made of amphiphiles with a chiral head group, a secondary amide group, and long alkyl chains. Their uniform helical pitches or rod and tubule diameters suggest uniform molecular conformations within the curved supramolecular assemblies. These conformations are characterized by chemical shift patterns in CPMAS ^{13}C-NMR solid state spectra. The assignment of such spectra obtained for microcrystals and lyophilized fibrous assemblies of five *N*-octylhexonamides is based on comparisons with (i) known single crystal structures, (ii) unequivocally assigned ^{13}C-NMR solution spectra, and (iii) application of established *gauche* effects. The crystal structure data may be taken from single crystal structures of corresponding amphiphiles, which are notoriously difficult to obtain, or from nonamphiphilic analogues. The procedure also detects crystal disorders. It is generally applicable to assemblies of molecules with at least four interconnected carbon atoms in a uniform conformation.

Introduction

Strong hydrogen bond chains between the head groups of fibrous molecular bilayers may mold all the molecules of such assemblies into one single conformation. In this sense, the supramolecular assembly then becomes "crystalline". Sometimes, this crystallinity is revealed in light and electron micrographs by the appearance of long-lived, rigid helices with a uniform pitch; sometimes one observes uniform tubules or ribbons.[1-7] In all such cases, the fibers can usually not be aligned and do not lend themselves to detailed X-ray or electron diffraction studies. The crystallinity of the fibers therefore remains an intelligent guess and the actual molecular conformations cannot be determined. Chemical shifts in CPMAS ^{13}C-NMR solid state spectra, however, depend on molecular conformations,[8-13] and single crystal structures of many compounds are known, which contain the chiral head group arrangements of the building blocks (=synkinons) of supramolecular assemblies. We here describe a procedure which applies the knowledge of molecular conformations in 3D-crystals to assign ^{13}C-signals to individual carbon atoms. The assigned conformations and chemical shifts are then applied to assign the solid state NMR spectra of lyophilized fibers and microcrystals of unknown structure (Figure 1). Crystal disorder and the arrangement of crystal sheets are also characterized.

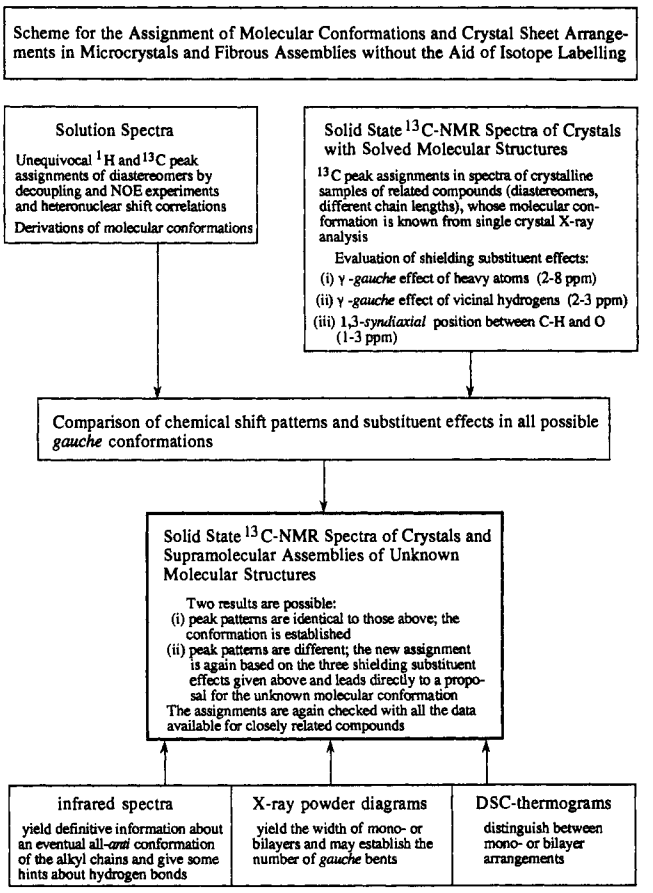

Figure 1. Scheme of conformational analysis based on crystal structures, CPMAS ^{13}C-NMR spectroscopy and various other techniques.

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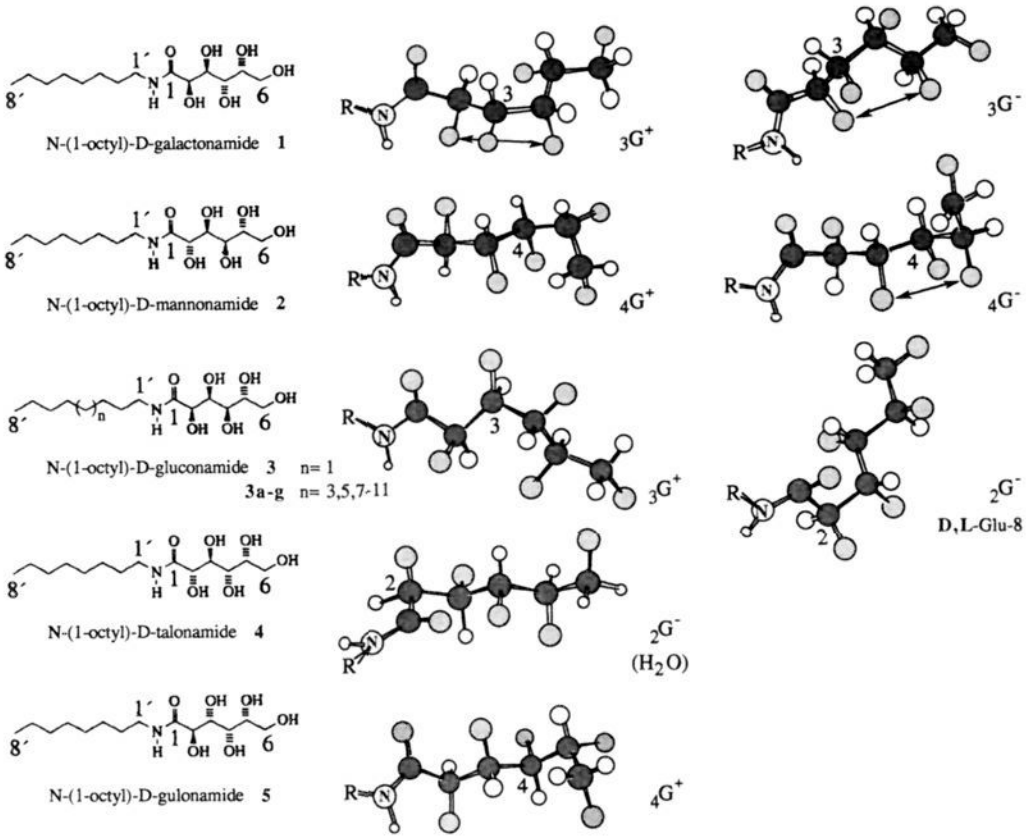

Figure 2. Structural formula and ball and stick models of the hexonamide conformations discussed in this paper.

Experimental Section

Synthesis of *N*-(1-Octyl)-D-hexonamides. Aminolysis of the hexonic acid lactones (Sigma) was performed by refluxing them with an equimolar amount of 1-aminooctane (Fluka) in methanol.[2,3] The products were crystallized from methanol and characterized by IR, NMR, and mass spectra as well as elemental analysis.

Measurements. The samples used for CPMAS ^{13}C-NMR solid state spectra consisted of 200 mg of hexonamide crystals or lyophilized fibers. The fibers were produced by rapid cooling of a 10% (w/v) aqueous gel in liquid nitrogen followed by freeze-drying. NMR spectra were recorded with a Bruker AC 250 spectrometer equipped with the adequate double air bearing CPMAS attachments at spinning rates of 4000 rotations per second. Glycine (δ = 176.1 ppm) was used as external standard. For IR and DSC measurements a Nicolet 5 SXC spectrometer and a Perkin-Elmer DSC-2C differential scanning calorimeter were used. X-ray diffraction diagrams of crystalline samples were performed with Ni-filtered Cu Kα radiation at a Philips PW 1050 powder diffractometer.

Results and Discussion

NMR Chemical Shift Assignments of Carbohydrate Head Groups with Known Conformation in Crystals. As a first step of conformational analysis, we chose the tentative assignment of ^{13}C-NMR signals obtained by CPMAS spectroscopy of crystals whose X-ray structure is known. Three shielding substituent effects causing upfield shifts were evaluated: (i) the *γ-gauche* effect of heavy atoms (2−8 ppm),[8,9] (ii) the *gauche* effect of vicinal hydrogen atoms (2−3 ppm),[10,11] and (iii) the shielding of carbon atoms bearing a hydrogen in 1,3-*syndiaxial* position to an oxygen atom (1−3 ppm).[12,13]

We exemplify such qualitative assignments with the carbohydrate head groups of *N*-octyl-D-glucon-, -talon-, and -gulonamides **3**−**5** (Figure 2). The gluconamide **3** shows an all-*anti* conformation of the carbon chain, a 2,4-*syndiaxial* repulsion between hydroxy groups, and a *gauche* oriented terminal oxygen atom in the crystal structure[14] (Figures 2 and 3). Correspondingly, five solid state ^{13}C-NMR signals for the carbon atoms bearing hydroxy groups are observed (Figure 4, Table 1). The signals at the high- and low-field sides should come from the primary alcohol C-6 carbon atom and the C-2 carbon neighboring the carboxamide group. Two signals are very close to each other and are assigned to carbon atoms C-4 and C-5, because their sterical environment is similar; the neighboring carbon atoms are in *anti* positions and both atoms possess one *γ-gauche* interaction to an oxygen atom. Furthermore, the vicinal hydrogen atoms are one in *gauche* and one in *anti* positions to 4-H and 5-H. Carbon atom C-3, on the other hand, is more shielded because its methine proton has two vicinal hydrogen atoms in *gauche* positions, whereas the positions of neighboring carbon and oxygen atoms are similar to those found for C-4 and C-5 (see Newman projections in Figure 3 and ball and stick model in Figure 2). We therefore assign the signal at higher field (70.5 ppm) to C-3. The molecular structure in single crystals of *N*-isopropyl-D-gluconamide (D-Glu-Iso),[15] which is not amphiphilic and thus much easier to crystallize than long chain derivatives, reveals the same all-*anti* conformation as the octyl amide **3** and produces a virtually identical CPMAS ^{13}C-NMR spectrum (Figure 4). The assignments therefore remain the same, although the crystal's symmetry and the strength of the amide hydrogen bond (see carbonyl carbon signals) are different in both crystals.

N-Octyl-D-gulonamide **5** crystallizes in the $_4G^+$ sickle conformation,[16] indicating a 120° counterclockwise rotation around the C-4−C-5 bond of the all-*anti* conformation.[17] The 3,5-

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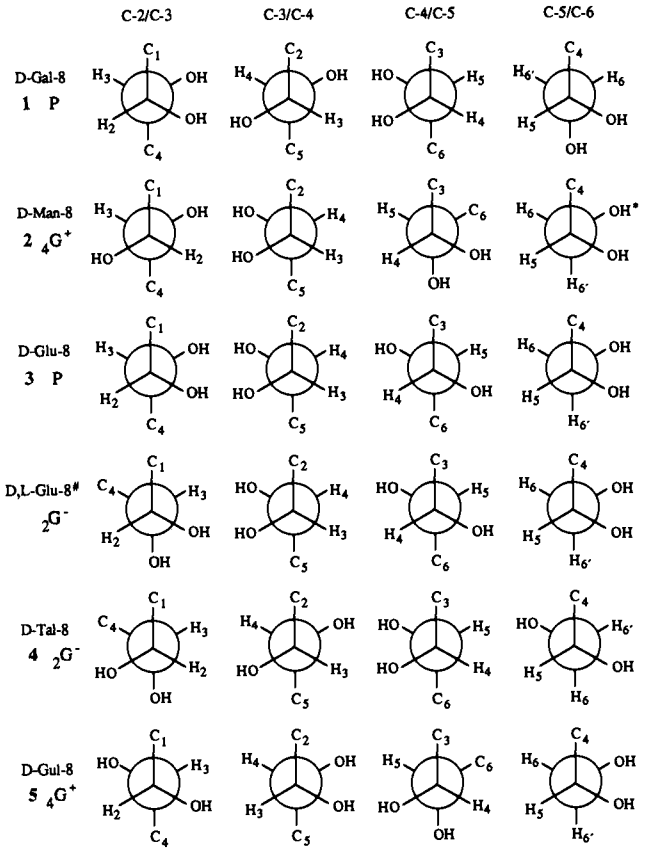

Figure 3. Newman projections of the carbon atoms C-2 to C-6 in the determined conformations of *N*-octyl-D-hexonamide **1-5** and D,L-gluconamide crystals. (*OH and H_6 may be interchanged. #The projections are related to the D-enantiomer.)

syndiaxial repulsion between hydroxy groups is removed by a *gauche* bend between C-3 and C-6. All four secondary alcohol carbon atoms are now in a similar chemical environment: the signal of C-2 neighboring the carboxamide carbon C-1 is shifted upfield because 2-H and O-4 are in a shielding 1,3-*syndiaxial* position. The downfield position of the C-3 resonance peak (72.8 ppm) results from the replacement of a C-3/O-5 by a less shielding C-3/C-6 *gauche* interaction, as compared to gluconamide **3**. The C-4 peak, on the other hand, should occur at highest field, because (i) O-2 and O-6 are *gauche* to it, (ii) 4-H and 3-H are *gauche* to each other, and (iii) there is a 1,3-*syndiaxial* repulsion between 4-H and O-2 (Figures 2 and 3). We therefore deduce the peak assignments C-3, C-2, C-5, and C-4 going from low to high field. The signals are, however, situated in a narrow range of 2.2 ppm, and supporting evidence is needed for these assignments (see below and Table 1).

The third solved crystal structure[18] of a diastereomer is that of talonamide **4**. It contains a $_2G^-$ sickle conformation with a *gauche* bend between C-1 and C-4. The downfield signal at 73.9 ppm can safely be assigned to C-2, because the carbon atom is neighboring a carboxamide group and underlies none of the shielding effects, which are found for the three other carbon atoms C-3 to C-5. The upfield signal clearly originates from C-4 because it is the only carbon atom which is *gauche* to three nonhydrogen atoms, namely, C-1, O-2, and O-6. Furthermore, there is an 1,3-*syndiaxial* position between 4-H and O-2 and a *gauche* interaction between 4-H and 5-H. Carbon atoms C-3 and C-5 have comparable chemical environments. We assign the signal at higher field (67.9 ppm) to C-5, because

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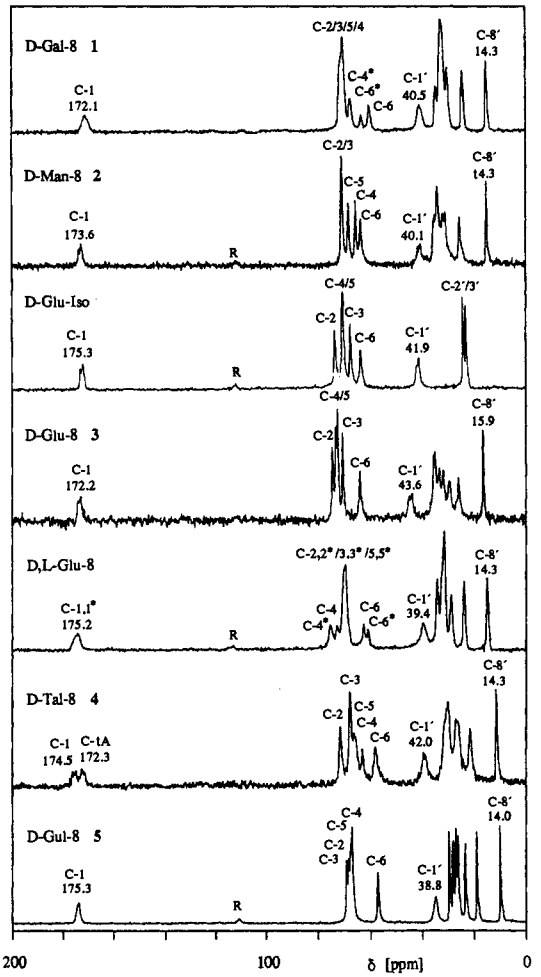

Figure 4. ^{13}C-NMR solid state spectra of crystals of *N*-octyl-D-hexonamides **1–5**, racemic D,L-gluconamide (D,L-Glu-8), and *N*-isopropyl-D-gluconamide (D-Glu-Iso) with peak assignments. The asterisks indicate peak splittings caused by a conformational disorder.

5-H is *gauche* to 4-H and to one of the methylene hydrogen atoms at C-6. The differences in chemical shifts between the carbon atoms C-2 to C-5 are relatively large in talonamide (total range: 8.6 ppm, Table 1) and thus reflect well the pronounced differences of *gauche* effects. The assignment C-2, C-3, C-5, and C-4 going from low to high field is therefore relatively safe and supported by comparisons with ^{13}C-NMR solution spectra (see next section).

Most confidence in the correctness of the tentative peak assignments comes from the fact that different head group conformations produce clearly differentiable patterns of carbon signals, as always exactly predicted by the three aforementioned rules. The same observation was made with the ^{13}C-NMR spectra of the hexonamides **1–5** in DMSO solution.[19] The peak assignments were deduced from ^{1}H-NMR spectra and converted to ^{13}C-NMR spectra by two-dimensional heteronuclear shift correlation experiments. Table 1 lists the main results of this procedure, namely, chemical shifts and assignments, together with the major conformational features. Since the peak assignments are based on multiple decoupling experiments of vicinal OH and CH protons, they are unequivocal. The conformational assignments based on the evaluation of coupling constants are tentative. The structural conclusions from chemical shifts and coupling constants in solution are then compared to the results from the CPMAS ^{13}C-NMR solid state spectra of crystalline

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Table 1. ^{13}C-NMR chemical shifts, shift differences, peak assignments, and major conformational features of the carbohydrate head groups of *N*-octyl-D-hexonamides **1**−**5** in DMSO-d_6 solution; crystals and lyophilized fibers of **1**−**5**, crystals of racemic octyl D,L-gluconamide, and *N*-isopropyl-D-gluconamide obtained from water. The chemical shifts of the galacton- and mannonamide **1** and **2** fibers are the same as found in the crystals. Unequivocal assignments and conformations are shadowed gray

chemical shifts (ppm)

	D-Gal-8 **1**			D-Man-8 **2**			D-Tal-8 **4**						
	sol.	|Δ|	cryst.	sol.	|Δ|	cryst.	sol.	|Δ|	cryst.	|Δ|	fiber	|Δ|	cryst.[a]
C-1	173.2	1.1	172.1	173.5	0.1	173.6	172.3	0	172.3[b]	0.5	171.8[c]	1.5	170.3
C-2	71.0	0.4	70.6	71.9	0.9	71.0	73.0	0.9	73.9	0.4	73.5	0.9	74.3
C-3	70.8	0.2	70.6	70.5	0.5	71.0	72.9	**2.8**	70.1	0.3	69.8	**3.3**	73.1
C-4	69.2	**1.4**	70.6	70.3	**4.8**	65.5	69.9	**4.6**	65.3	**1.0**	64.3	**5.7**	70.0
		1.5	67.7										
C-5	69.9	0.7	70.6	70.9	**2.6**	68.3	70.3	**2.4**	67.9	**1.0**	66.9	**3.1**	70.0
C-6	63.3	2.9	60.4	63.8	0.2	63.6	62.9	2.5	60.4	**1.5**	58.9	**4.7**	63.6
		0.3	63.6										
	all-*anti*		all-*anti*	all-*anti*		*gauche* C-3/C-6 C-4/O-6 $_4G^+$	*gauche* C-1/C-4 $_2G^+$		*gauche* C-1/C-4 C-3/O-1 C-4/O-6 $_2G^-$		*gauche* reorient. of O-6 $_2G^-$		all-*anti*

[a] recrystallized from methanol; [b] and 174.5; [c] and 174.8

	D-Gul-8 **5**			D-Glu-8 **3**					D-Glu-iso		D,L-Glu-8	
	sol.	|Δ|	cryst.	sol.	|Δ|	cryst.	|Δ|	fiber	|Δ|	cryst.	|Δ|	cryst.
C-1	173.1	2.2	175.3	172.3	0.1	172.2	0.7	171.5	3.1	175.3	3.0	175.2
C-2	71.8	0	71.8	73.7	**0.9**	74.6	**3.3**	71.3	0.8	75.4	**4.6**	70.0
C-3	72.8	0	72.8	70.2	0.3	70.5	0.8	71.3	1.3	69.2	0.5	70.0
C-4	69.5	**1.1**	70.6	72.5	**0.7**	73.2	**1.3**	74.5	1.1	72.1	0.2	73.4
											2.9	76.1
C-5	73.1	**2.0**	71.1	71.5	**1.1**	72.6	**1.3**	71.3	0.5	72.1	**2.6**	70.0
C-6	62.5	1.7	60.8	63.4	0.5	63.9	0.1	63.8	1.3	65.2	1.0	62.9
											2.8	61.1
	"all-*anti*" highly flexible		*gauche* C-3/C-6 $_4G^+$	*gauche* C-3/C-6 flexible $_4G^+$		all-*anti* C-4/O-6		*gauche* C-1/C-4 C-4/O-6 $_2G^-$		all-*anti* C-4/O-6		*gauche* C-1/C-4 $_2G^-$

material. In the latter case, the molecular conformations are firmly established, but the peak assignments are not certain (see above). The comparison of solution and solid state NMR spectra, however, immediately shows that the molecular conformations of the diastereomers **1**−**5** in solution are different and as variable as in the solid state, although the spread of signals in the solution spectra is smaller by a factor of about 2. The all-*anti* conformations of galacton- and mannonamides **1** and **2**, for example, lead to similar chemical environments of all carbon atoms, and the signal spread of secondary alcohol carbon atoms C-2 to C-5 is correspondingly small (total range: 1.8 ppm, Table 1). Deviations of conformations are again reflected in different peak patterns.

In Table 1, Δ values corresponding to chemical shift differences between ^{13}C-NMR signals in solution and solid state spectra are given. They range from 0 to 5 ppm. The absolute Δ values have little meaning, because they often only reflect different flexibilities of the dissolved hexonamide monomers. Large deviations from an average Δ value of a given diastereomer, however, are taken as an indication of a change from one conformation in solution to another in the solid state. The conversion of an all-*anti* to a $_4G^+$ sickle conformation, for example, always leads to remarkable shift differences for the C-4 and C-5 resonance peaks. This can be seen if one compares the solid state spectra of diastereomers, whose crystal structures are known, and it also becomes evident in comparisons between assigned solution and solid state spectra. No conformational change remains undetected. No solution or solid material with the same molecular conformation produced different sequences of ^{13}C-NMR signals. There is, however, one pitfall. The C-2 signal of the gluconamide **3** in solution, for example, cannot be considered in such an analysis because all of the staggered conformations are equally likely at the C-2−C-3 axis.[19] It is not helpful to apply chemical shift values of molecules or parts of them when there is no preferred conformation. The *gauche* bend of gluconamide **3** in solution and the all-*anti* conformation in the crystal is, however, clearly evident from the relatively larger shifts of the C-4 and C-5 signals.

All observed similarities and differences between chemical shifts can thus be explained by the comparison of experimentally determined NMR solution and solid state spectra and by application of the *γ-gauche* effect of heavy atoms, the *gauche* effect of vicinal hydrogen atoms, and the carbon shielding 1,3-*syndiaxial* position of a hydrogen and an oxygen atom. Not a single contradiction between tentative peak assignments in the solid state and the qualitative application of these rules to the conversion of solution to solid state data was detected.

Chemical Shift Assignments of Carbohydrate Head Groups with Unknown Conformation in Crystals and Fibers. ^{13}C-NMR solid state spectroscopy becomes, however, most useful if it succeeds in assigning conformations to compounds that cannot be converted to single crystals. Galacton- and mannonamides **1** and **2**, for example, withstood all attempts toward single crystal formation. A comparison of the solution and solid state spectra of galactonamide **1** immediately reveals the same peak patterns corresponding to an all-*anti* conformation of the molecules, in consideration of a splitting of the C-4 and C-6 peaks in the crystal's spectrum (Table 1). In the spectra of mannonamide **2**, on the other hand, strong deviations of the chemical shifts of C-4 and C-5 can only be explained with a $_4G^+$ sickle conformation in the crystals, similar to the gluconamide **3** conformation in solution (Table 1). The alternative $_4G^-$ sickle can obviously not be formed because it would contain a repulsive O-3/O-5 interaction (Figure 2). A *gauche* bend in a mannonic acid head group is surprising with regard to the possibility of forming sterically undisturbed all-*anti* conformation. To our knowledge so far, only one compound with comparable sickle conformation has been described, namely, D-glycero-D-*manno*-heptitol.[20] One must assume that a favorable *γ-gauche* effect of neighboring oxygen atoms enforces bending here. In the galactonamide diastereomer this bending is presumably hindered by the unfavorable formation of a 1,3-*syndiaxial* oxygen pair (Figure 2).

The molecular conformation in crystals of racemic octyl D,L-gluconamide (D,L-Glu-8) could also be resolved by ^{13}C-NMR solid state spectroscopy (Figures 2−4). Apart from a splitting of the signals for C-4 and C-6, one observes strong deviations for the C-2 chemical shift as compared to the D-enantiomer. Analysis of the limited number of conformations at C-2 clearly indicates a $_2G^-$ conformation with a *gauche* bend between C-1 and C-4. This conformation was also found in crystals of gluconic acid,[21] glucitol,[22] and odd-numbered 1-deoxy(*N*-methylalkanamido)-D-glucitols.[23,24]

It is now also possible to evaluate the conformations within the fibers of the hexonamides **1**−**4**. Lyophilized fibers were used instead of aqueous gels, because aqueous suspensions usually lead to technical problems at spinning rates of about 4000 rotations per second. Only in the case of gluconamide **3**, was it therefore shown that solid state spectra of suspended and lyophilized helices were identical. Furthermore, all fibers produced identical electron micrographs for freshly prepared gel samples and lyophilized fibers resuspended in water. In the case of the lyophilized gluconamide **3** helices, one observes a strong shift deviation between fiber and crystal solid state spectra for C-2, which can be traced back to the same $_2G^-$ conformation observed in the racemic crystals. In the fiber,

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however, the carboxamide carbon is found at 171.5, in the racemate at 175.2 ppm. This is about the same difference which is found between *N*-octyl-D-gluconamide (172.2 ppm) and *N*-isopropyl-D-gluconamide (175.3 ppm) and results from different amide hydrogen bond lengths.[14,15] Shift differences of the C-4 and C-5 signals result from a reorientation of the terminal hydroxy group from a *gauche* (crystal) to an *anti* (fiber) position with respect to the carbon chain (Table 1). This reorientation also causes the shift differences in the fiber and crystal spectra of talonamide **4**. The molecular $_2G^-$ conformation, however, is the same in fibers and in crystals. The CPMAS ^{13}C-NMR spectra of lyophilized fibers of galactonamide **1** (twisted ribbons) and mannonamide **2** (rolled up bilayers) are also identical with those of the crystals.

The common features of the NMR spectra can now be summarized as follows: (i) the spreading of ^{13}C-signals in solution spectra is small if the corresponding carbon chain is flexible, and large if it is rigid; (ii) the *γ-gauche* effects determine most of the conformations found in solution and with some restrictions also in crystals; (iii) ^{13}C-NMR chemical shifts in solution and solid state spectra correspond to each other; and (iv) crystal packings or symmetries have no important effect (the head group conformations in bilayer crystals and in supramolecular assemblies in water are usually the same). If, however, the molecules are head-to-tail oriented (gluconamide crystals), the usual *gauche* bend disappears and an all-*anti* conformation occurs.

A preliminary conclusion on the influence of molecular conformations on the curvature of fibers, which are held together by amide hydrogen bond chains, can also be drawn: a bend close to the fiber's surface ($_4G$) has little effect (mannonamide), whereas a bend close to the amide bond and the hydrophobic core ($_2G$) causes large curvature (glucon- and talonamide; Figure 2). It is possible that the $_4G$ bend allows more room for hydration of a fiber than the $_2G$ bend.

Conformational Polymorphism and Crystallographic Disorder. The determination of molecular conformations of carbohydrate head groups in crystals and fibers by ^{13}C-NMR solid state spectroscopy, based on crystal structures, is not the only merit of this method. Mixtures of conformers and polymorphism can also be detected and analyzed. We give three examples. In cases where single crystal structures have been solved, one may find crystallographically independent molecules within an asymmetric unit. In talonamide **4** crystals, obtained from water solutions, for example, two molecules with very similar $_2G^-$ sickles are present which produce hydrogen bonds of 303 and 349 pm lengths.[18] These different molecular interactions are also reflected in the CPMAS ^{13}C-NMR spectra, in which two carbonyl carbon signals at 172.3 and 174.5 ppm appear. If the same talonamide is crystallized from methanol, only one signal at 170.3 ppm is found. The peak pattern of carbon atoms C-2 to C-5 indicates the conversion from the $_2G^-$ sickle (water) to the all-*anti* (methanol) conformation inclusive of O-6. An abolition of *gauche* interactions between C-4 and C-1/O-6 as well as reorientations between the hydrogen atoms at C-5 and C-6 lead to downfield shifts of the participating signals and result in the observed peak pattern (Table 1). It follows that talonamide **4** crystallizes in two similar $_2G$ sickle conformations with different amide hydrogen bonds from water and as uniform all-*anti* conformer from methanol. This solvent-dependent conformational polymorphism in open-chain carbohydrates has so far only been described for potassium gluconate monohydrate.[25] In polymorphic alditols, one only finds differences of the hydrogen bond patterns, which cause small ^{13}C-shifts of ±0.5 ppm.[26,27]

In the solid state spectrum of galactonamide **1** crystals, two signals for C-6 at 60.4 and 63.6 ppm are found, in an intensity ratio of approximately 1:2 (Figure 4). Although there is no *quantitative* relationship between peak area and number of carbon atoms, because of the cross polarization and broad band hydrogen decoupling conditions, a comparison of signal intensities justifies a *qualitative* estimate. The splitting cannot be caused by crystallographically independent molecules, because they should produce a 1:1 signal. In DMSO solution, only one signal at 63.3 ppm appears and has been assigned to a rotating terminal hydroxy group with preferred *gauche* orientation of O-6 to the carbon chain.[19] The upfield signal at 60.4 ppm thus represents the *anti* orientation of O-6, which obviously is preferred in the crystals. The different oxygen orientations also cause a splitting of the C-4 signal, which produces a new signal at 67.7 ppm and a downfield part overlapped by the main peak at 70.6 ppm. Galactonamide **1** crystals thus combine the two terminal oxygen orientations, which have been found in galacticol (*anti*)[28] and 1-deoxy-1-nitro-D-galacto-hexitole (*gauche*).[29] A similar disorder of terminal oxygen atoms leads to the peak splitting in the spectrum of the racemic D,L-gluconamide (Figure 4) and has also been detected in single crystals of the related bolaamphiphile *N*-[8-(D-gluconamido)octyl]-D-gluconamide.[30]

Alkyl Chain Conformations and Crystal Sheet Arrangements. The alkyl chain conformations of acyclic amphiphiles can in general not be safely analyzed by CPMAS ^{13}C-NMR spectroscopy, because the differences in chemical shifts are too small. Specific labeling of each carbon atom or carbon atom pairs would be required for such an analysis. Fortunately, it has been shown by infrared spectra (see next section) and single crystal X-ray diffraction that the alkyl chains are always all-*anti* configured, except for the N−C-1′−C-2′−C-3′ sequence, which is often *gauche*. Here, the chemical shift of the easily assigned C-1′ signal gives some hint. It occurs at 43−44 ppm if the methylene group next to the nitrogen atom is part of an *anti* sequence (glucon- and talonamide crystals;[14,18] Figure 4, Table 2), whereas a *gauche* bend causes an upfield shift of 4−5 ppm (gulonamide and odd-numbered gluconamide crystals).[16,31,32] Moreover, this *gauche* bend was deduced from the ABMX signal of C-1′ in the ^{1}H-NMR spectrum of gluconamide **3** dissolved in DMSO.[19] Consequently, the corresponding ^{13}C solution signal appears at 38.3 ppm.

There is a second alkyl chain peak position of diagnostic potential: the chemical shift of the terminal methyl group (C-8′) can always be taken as a good indicator of its chemical environment and thus of the sheet arrangements. A value of about 14 ppm always indicates tail-to-tail arrangement of molecules in crystals or supramolecular assemblies; a value above 15.5 ppm means head-to-tail oriented crystal sheets. This is true for diastereomers (Table 2) and homologues (Figure 5).

Thus, comparisons of solid state spectra of homologous compounds give clear evidence for deviations within crystal structures. For example, the spectrum of tetradecyl gluconamide **3c** reveals the same peak pattern and chemical shifts as the octyl homologue **3**, indicative of identical molecular orientation and conformation (Figures 4 and 5). In contrast, the chemical shifts

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Table 2. ^{13}C-NMR chemical shifts of the alkyl chains of *N*-octyl-D-hexonamides **1**–**5** in crystals. The peak positions in DMSO-d_6 solution and lyophilized fibers are the same within the experimental error

	chemical shifts (ppm)					
	1	**2**	**3**	**4**	**4**[a]	**5**
C-1′	40.5	40.1	43.6	42.0	40.7	38.8
C-2′	31.9	33.6	34.5	32.9	33.0[b]	32.2
C-3′[c]	29.5	30.5	29.1	29.8	29.8	27.5
C-4′[c]	31.9	31.4	31.4	32.9	31.7	30.3
C-5′[c]	31.9	33.6	33.0	32.9	31.7	31.1
C-6′	34.0	34.6	34.5	32.9	34.4[b]	33.6
C-7′	23.6	24.9	25.4	24.4	24.3	23.2
C-8′	14.3	14.3	15.9	14.3	14.4	14.0

[a] Recrystallized from methanol. [b] Assignments may be interchanged. [c] Assigned in analogy to Bull et al.[46]

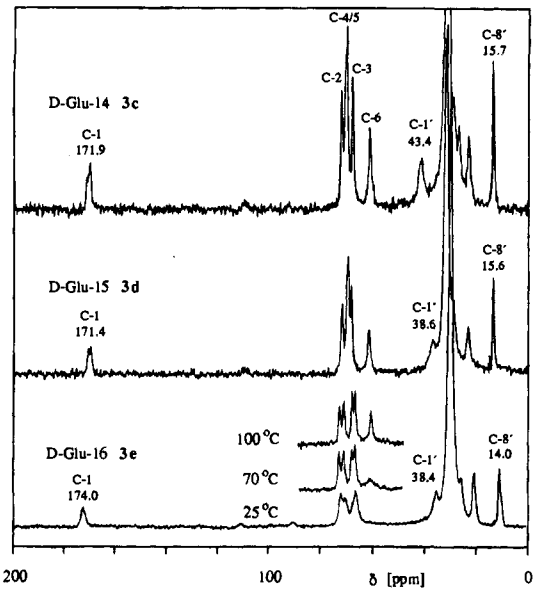

Figure 5. ^{13}C-NMR solid state spectra of *N*-alkyl-D-gluconamide crystals with tetradecyl (**3c**), pentadecyl (**3d**), and hexadecyl (**3e**) chain at 25 °C. In addition the head group peak patterns of **3e** at 70 °C and 100 °C are shown.

of the α-methylene and methyl carbon atom signals (C-1′ and C-8′) in the hexadecyl gluconamide **3e** spectrum point to the bimolecular orientation of monomers with *gauche* bend at the amide group. The head group peak pattern fits that found for octylamide **3** after heating to about 80 °C and corresponds to the $_3G^+$ sickle conformation.[7] At 25 °C the signal of carbon atom C-6 is rather broad and weak, caused by slow rotation of the hydroxymethyl group with respect to the NMR time scale. With increasing temperature and enhanced rotational mobility, the C-6 signal appears at a chemical shift indicative of a preferred *anti* oriented terminal oxygen atom O-6. Pentadecyl gluconamide **3d** exhibits an intermediate behavior, its orientation and head group conformation correspond to the tetradecylamide **3c**, whereas the peak position of C-1′ matches that of the hexadecylamide **3e**. We may therefore conclude that CPMAS ^{13}C-NMR spectra allow a complete analysis of molecular structures provided that some closely related crystal structures are known and that the NMR solution spectra allow decoupling experiments.

Supporting Evidences for the Assignments of Conformations and Crystal Sheet Arrangements. (a) Infrared Spectra. Infrared spectra cover the ultrashort time scale of 10^{-13} s and reflect *anti-gauche* ratios independently of molecular rotations.[33]

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Table 3. Infrared wavelengths of characteristic absorption frequencies in the spectra of crystalline *N*-octyl-D-hexonamides **1**–**5**

	wavelengths (cm^{-1})				
	1	**2**	**3**	**4**	**5**
ν(O-H)	3385/3282[a]	3387/3325 3233	3531/3389[a] 3358[a]	3503/3438 3394/3296	3425/3330[a]
$\nu_s(CH_2)$	2851	2850	2851	2851	2852
amide I	1650	1639	1646	1657/1611	1624
amide II	1556	1555	1529	1546/1513	1539
$\delta(CH_2)$[b]	1467	1467	1472/1462	1478/1468	1464
$\delta(CH_2)$[c]	1268–1209	1296–1205	1272–1222	1277–1224	1289–1244

[a] Broad signals. [b] $\delta(CH_2)$ scissoring band. [c] $\delta(CH_2)$ progression of wagging vibrations.

The most important result of comparative IR spectroscopy in the present case is that all ν(C-H) stretching absorptions and the progression of $\delta(CH_2)$ wagging vibrations of the octyl chains are identical in the spectra of hexonamide **1**–**5** crystals (Table 3) and in lyophilized fibers. In particular, the $\nu_s(CH_2)$ band around 2851 cm^{-1} and the $\delta(CH_2)$ "fingerprint" signals between 1205 and 1296 cm^{-1} indicate the same all-*anti* conformation of all octyl chains.[33–37] The $\delta(CH_2)$ scissoring band around 1470 cm^{-1} is extremely sensitive for monitoring the alkyl chain packing. Owing to a crystal field effect resulting from interchain interactions between all-*anti* chains, the band splits into two clearly resolved singularities. But this only occurs when the alkyl chains are packed in an orthorhombic or monoclinic crystal lattice, whereas hexagonal or triclinic packings are indicated by a single $\delta(CH_2)$ scissoring mode.[38–41] Consequently, the monoclinic $P2_1$ and orthorhombic $P2_12_12_1$ crystal lattices[14,18] of glucon- and talonamides **3** and **4** lead to splittings at 1462/1472 and 1468/1478 cm^{-1}, respectively. In gulonamide **5** crystals,[16] the octyl chain mobility prevents any band splitting, although the molecules crystallize in the monoclinic C2 space group. Only if the alkyl chain mobility is hindered, as in the homologous hexadecyl gulonamide, does the splitting appear at 1464/1474 cm^{-1} (data not shown). Thus the strong peak at 1467 cm^{-1} in the mannonamide **2** spectrum suggests a triclinic packing of octyl chains, in accordance with the crystal structures of odd-numbered gluconamides. On the other hand, a small low-frequency shoulder at the scissoring band of galactonamide **1** (1467 cm^{-1}) probably indicates an orthorhombic or monoclinic crystal lattice.

The strength of conformational and packing arguments derived from the infrared spectra has no match in the carbohydrate region. Nevertheless, a look at amide I and II absorption bands is instructive. The different amide hydrogen bond lengths of the crystallographically independent talonamide **4** monomers (303 and 349 pm with N–H···O bond angles of 156 and 147°) produce two amide I and II bands each at 1611/1657 and 1513/1546 cm^{-1}. The amide I absorptions of gluconamide **3** (1646 cm^{-1}) and gulonamide **5** (1624 cm^{-1}) can be traced back to N···O distances of 305 and 287 pm with bond angles of 150 and 136°. Thus the amide I frequencies of galacton- and mannonamides **1** and **2** at 1650 and 1639 cm^{-1}, respectively,

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Table 4. Crystal-to-crystal rearrangement temperatures and enthalpies of *N*-alkyl-D-hexonamide crystals containing different chain lengths. High enthalpy values, indicating a head-to-tail sheet arrangement within the crystals, are shadowed gray

		T_{rearr} (°C)	ΔH_{rearr} (kJ/mol)			T_{rearr} (°C)	ΔH_{rearr} (kJ/mol)
D-Gal-8	1	a	a	D-Glu-10	3a	88	15.0
D-Man-8	2	64	2.5	D-Glu-12	3b	94	17.7
D-Glu-8	3	86	13.3	D-Glu-14	3c	99	20.0
D-Tal-8	4	81/83/	2.2/2.5/	D-Glu-15	3d	92/101	5.5/1.6
		102	3.5	D-Glu-16	3e	104	3.5
D-Gul-8	5	115	1.0	D-Glu-17	3f	102	2.7
				D-Glu-18	3g	108	3.0

[a] Values not accessible, because of a continuous enthalpy gain.

should indicate the same amide hydrogen bond geometry as in gluconamide crystals. The hydroxy hydrogen bonds produce ν(O−H) stretching vibrations between 3300 and 3500 cm^{-1}. In the spectrum of crystals of gluconamide **3**, the peaks at 3358 and 3389 cm^{-1} should represent the homodromic cycle[14] between adjacent molecules, whereas the high-frequency absorption at 3531 cm^{-1} results from the free 2-OH hydroxy group.[41] Similar peak positions at 3330 and 3425 cm^{-1} in the gulonamide **5** spectrum support the assumed cyclic hydrogen bond scheme here too.

(b) Differential Scanning Calorimetry. Differential scanning calorimetry data give no information about molecular conformations but support the conclusions about crystal sheet arrangements derived from the chemical shift of the methyl group signal in the CPMAS NMR spectra. The DSC data of the bilayer crystals of octylamides **1**, **2**, **4**, and **5** reveal only small signals for endothermic processes with enthalpies between 1.0 and 4.8 kJ/mol in addition to the melting peak. In contrast, the endothermic enthalpies of the monolayer crystals of gluconamide **3** as well as the homologues **3a−c** with decyl, dodecyl, and tetradecyl chains amount to 15.0−20.0 kJ/mol. Longer alkyl chains (hexadecyl, heptadecyl, and octadecyl: **3e−g**) again cause low enthalpies between 2.7 and 3.5 kJ/mol (Table 4). The DSC thermograms thus clearly differentiate between a molecular bilayer arrangement (low enthalpy) and head-to-tail arrangement (high enthalpy). The pentadecyl gluconamide **3d** represents the border case between both molecular orientations. The melting points of the homologous gluconamides are insensitive to the sheet arrangement; they decrease from 158 to 148 °C with increasing alkyl chain lengths, whereas the melting enthalpies rise from 41.5 to 59.1 kJ/mol.

(c) X-ray Diffraction. Finally it should be noted that the molecular orientation and, with some restriction, conformation of hexonamides **1−5** are also detectable by X-ray diffraction measurements of the same samples used for the solid state NMR spectroscopy. One *gauche* bend shortens a molecule by approximately 0.12 nm, compared to the all-*anti* conformation.[42] The small-angle reflections in the diffraction patterns of the octylamide crystals thus always indicate the bimolecular arrangements with the exception of the head-to-tail oriented gluconamide **3** (Table 5). Gulonamide **5** bilayers reveal a spacing between lattice planes of 3.46 nm, corresponding to three *gauche* bends in each half-layer, which were also found in single crystals and solid state NMR spectra. Exactly the same spacing (3.45 nm) in the diffraction diagram of mannonamide **2** supports the NMR interpretation of a $_4G^+$ sickle conformation with additional *gauche* bends at the amide group and the terminal O-6 atom. A replacement of one *gauche* arrangement (and two within the bilayer) by *anti* oriented atoms, as it was found for talonamide **4**, increases the effective molecular length by 0.12 nm and results in a spacing of 3.58 nm. A second rearrangement producing the proposed *anti* orientation of the terminal O-6 of galactonamide **1** rises from the spacing to the experimentally found 3.70 nm (Table 5).

Table 5. Small-angle (d) and wide-angle (d_x) reflections and half-widths ($h_{1/2}$) of *N*-octyl-D-hexonamide **1−5** crystals measured at room temperature

	d (nm)	d_x (nm)	$h_{1/2}$ (°2θ)
D-Gal-8 **1**	3.70	0.44	0.6
D-Man-8 **2**	3.45	0.44	0.4
D-Glu-8 **3**	1.79[a]	0.45	0.3
D-Tal-8 **4**	3.58	0.44	0.4
D-Gul-8 **5**	3.46	0.44	0.8

[a] Head-to-tail arrangement.

The X-ray diffraction patterns also produce wide-angle reflections, whose positions and widths give evidence for packing densities and conformations of the alkyl chains. For lecithins a sharp reflection at 0.42 nm has been related to an all-*anti* conformation and a broad signal at 0.45 nm to more fluid alkyl chains.[43,44] Thus the wide-angle reflection at 0.44 nm in the patterns of the octylamide **1−5** crystals would suggest fluid alkyl chains, but this is not compatible with the small half-widths (see Table 5) and the extended octyl chain conformations found in the single crystals of **3−5**. Obviously the wide-angle reflections in lecithins with long alkyl chains (≥C14) appear in general at lower d_x values than in octyl derivatives.

Conclusion

In summary, we have demonstrated the potential of CPMAS ^{13}C-NMR solid state spectroscopy of microcrystalline samples and lyophilized supramolecular assemblies to solve molecular and crystal structures, if it is supported by infrared, calorimetry, and X-ray diffraction data. The results also show that the direct use of crystal structure data for the modeling of supramolecular assemblies is often not justified (see gluconamide **3** conformations in crystals and fibers).

The method presented is, of course, not restricted to carbohydrate assemblies. We determined, for example, the molecular conformation of the macrocyclic bolaamphiphile disodium 2,5,-20,23-tetraoxo-1,6,19,24-tetraoxacyclohexatriacontan-3,21(22)-diylbis(thio)bis(acetate)[45] with the same methodology and are applying it currently to amino acid bolaamphiphiles. We are certain, that this method can be successfully applied to other molecular assemblies, provided that they contain at least four interconnected carbon atoms in a uniform conformation. Obvious candidates are, for example, amphiphiles with amino acid or nucleic acid head groups as well as helical homopeptides and nucleotides.

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